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Studies on fungi in a pinewood soil
III. Fungal growth and total microbial activity

BY

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INTRODUCTION

Fungi in soil can exist in a variety of physiological states (actively growing hyphae, dormant spores, chlamydospores, sclerotia, resting mycelium, dead mycelium) of which only the actively growing hyphae will be important in the colonization, exploitation and exhaustion of the soil organic material. In previous papers (PARKINSON and BALASOORIYA, 1967 ; BALASOORIYA and PARKINSON, 1967) results of qualitative investigations on fungi in the soil at Freshfield (Lancashire) have been described. These results were obtained by techniques (washing techniques) designed to isolate fungi present in various soil microhabitats in the hyphal state. However, such washing techniques do not remove all spores and other propagules, therefore it cannot be said that all the fungi isolated originated from hyphae in the soil. Inability to obtain all fungi in soil samples into culture is another factor which introduces error to any attempt to assess « fungal activity » by means of culture studies. Thus the use of the soil washing technique, although it allows assessment of substrate relationships and the degree of substrate colonization, gives incomplete information on fungal activity.

As it is living mycelium which is physiologically active in soil, the determination of the quantity of such mycelium should provide an index of fungal

activity. A thorough discussion of the methods available for such a determination has been given (NICHOLAS and PARKINSON, 1967) and the agar film technique (JONES and MOLLISON, 1948) was shown to the most suitable of the existing techniques for absolute determinations of mycelial lengths in soil samples. However, the inability to distinguish accurately between living and dead mycelium, when using to assess fungal activity. This is not the only objection to the use of this technique for assessing fungal activity — the assumption that total mycelial length can be used as an accurate index of total « fungal activity » presupposes that the metabolic activity of different types of mycelium (as well as different portions of the same mycelium) is the same. This is an unlikely situation.

An accurate method of estimating « fungal activity » in soil would be to determine some component of their metabolic process, and the measurement of respiratory rate has been commonly used for this purpose. The introduction of the Warburg technique for measuring the oxygen uptake of soil samples (WEBLEY, 1947 ; QUASTEL and SCHOLEFIELD, 1951 ; 1953 ; ROVIRA, 1953) led to the regular use of this technique or some variant of it. A number of criticisms have been levelled at the use of this technique for assessing microbial activity — the degree of disturbance of the soil, the temperature which is normally used, the small samples of soil used, are some of these criticisms. The normal use of the Warburg technique allows determinations of total microbial respiration in soil to be made. The part played by fungi in this total activity is difficult to determine.

Although inadequacies are inherent in both the hyphal measurement technique (by the agar film technique) and in the measurement of total microbial activity by respirometry, it appeared that these were the best techniques presently available to provide supplementary information to the qualitative studies on fungal populations in the horizons of a pinewood soil at Freshfield, Lancashire. The results of these quantitative studies are presented here.

METHODS

a) *Soil Sampling:* Details of the soil under investigation have been given previously (PARKINSON and BALASOORIYA, 1967). At 2-monthly intervals over a period of 14 months the following sampling procedure was adopted: a pit approximately 1 sq. metre in area was dug, the four walls of the pit were scraped to remove contaminating soil, and samples of the mineral horizons were taken with sterile specimen tubes from depths of 7.5 cm., 15.0 cm., and 30.0 cm. (i.e. from the A_1 horizon and from 2 depths of the C horizon, these depths are subsequently referred to as C_1 and C_2). 20 samples were taken at each depth.

Samples from the layers of the litter horizon (i.e. L, F_1, F_2, and H layers) were collected in plastic bags from 20 places within the sampling area.

In the laboratory the 20 small samples from each horizon were bulked and thoroughly mixed. From each bulk sample, sub-samples were taken for determination of organic carbon content, determination of moisture content, measurement of hyphal length, and measurement of oxygen uptake.

b) *Measurement of hyphal length:* The method used was that of JONES and MOLLISON (1948) as modified by THOMAS, PARKINSON and NICHOLAS (1965). For the soil under study this technique was only applicable to samples from the H layer and from the A_1 and C horizons. Preliminary study showed that accurate measurements could be obtained when agar films were prepared using 0.5 g. samples of H layer material and 4.0 g. sub-samples of A and C horizon soil. Numerous slides were prepared for each horizon at each of the bimonthly sampling times. Measurements of hyphal lengths were made using the high power ($\times$ 40) objective of the microscope and camera lucida drawing of the hyphal fragments observed (these drawings were subsequently measured using a map-measuring device). Reproducible results were obtained by the examination of 100 randomly selected microscope fields on each of 3 different agar films.

c) *Measurement of oxygen uptake*: The procedure adopted was the Warburg technique using modified flasks (PARKINSON and COUPS, 1963). In order to try to reduce disturbance the soil samples were not sieved prior to their introduction into the flasks. The amount of soil or litter placed in each flask was accurately weighed. After introduction into the flasks the soil was allowed to « settle » for 18 hours at 25° C after which oxygen uptake measurements were made over a 5 hour period at 25° C.

d) *Determination of organic carbon*: This was done by oxidation with potassium dichromate and collection and weighing of the CO_2 evolved (SHAW, 1959). In the case of samples from the litter layers, these were ground to a fine powder before the estimations were made.

e) *Determination of soil moisture*: This was done by drying known weights of freshly sampled soil to constant weight at 105° C.

RESULTS

Measurement of total mycelial length.

The variations in the lengths of mycelium observed in the 4 horizons during the 14 month sampling period are shown in Figure 1. From this it can be seen that there were much wider seasonal variations in mycelial lengths in the mineral soil horizons than in the H layer of the organic horizon. All the mineral horizons showed a progressive decrease in mycelium from October to February, after which mycelial lengths rose. In the A_1 horizon, maximum mycelial content was recorded during the June-August period; in the upper C horizon (C_1) this maximum was in April; in the lower C horizon (C_2), maximum mycelial content was recorded in June. The variations in length of mycelium in the H layer, A horizon and upper and lower zones of the C horizon are given in Table 1. These values represent the means of seven sets for values obtained for each horizon (hence seasonal variation has been eliminated). The substantial seasonal variations recorded are responsible for the large standard deviations recorded. In accordance with the observations of previous workers (e.g. BROWN, 1958; NICHOLAS et al., 1965) there is an apparent decrease in the amount of mycelium with depth in the soil.

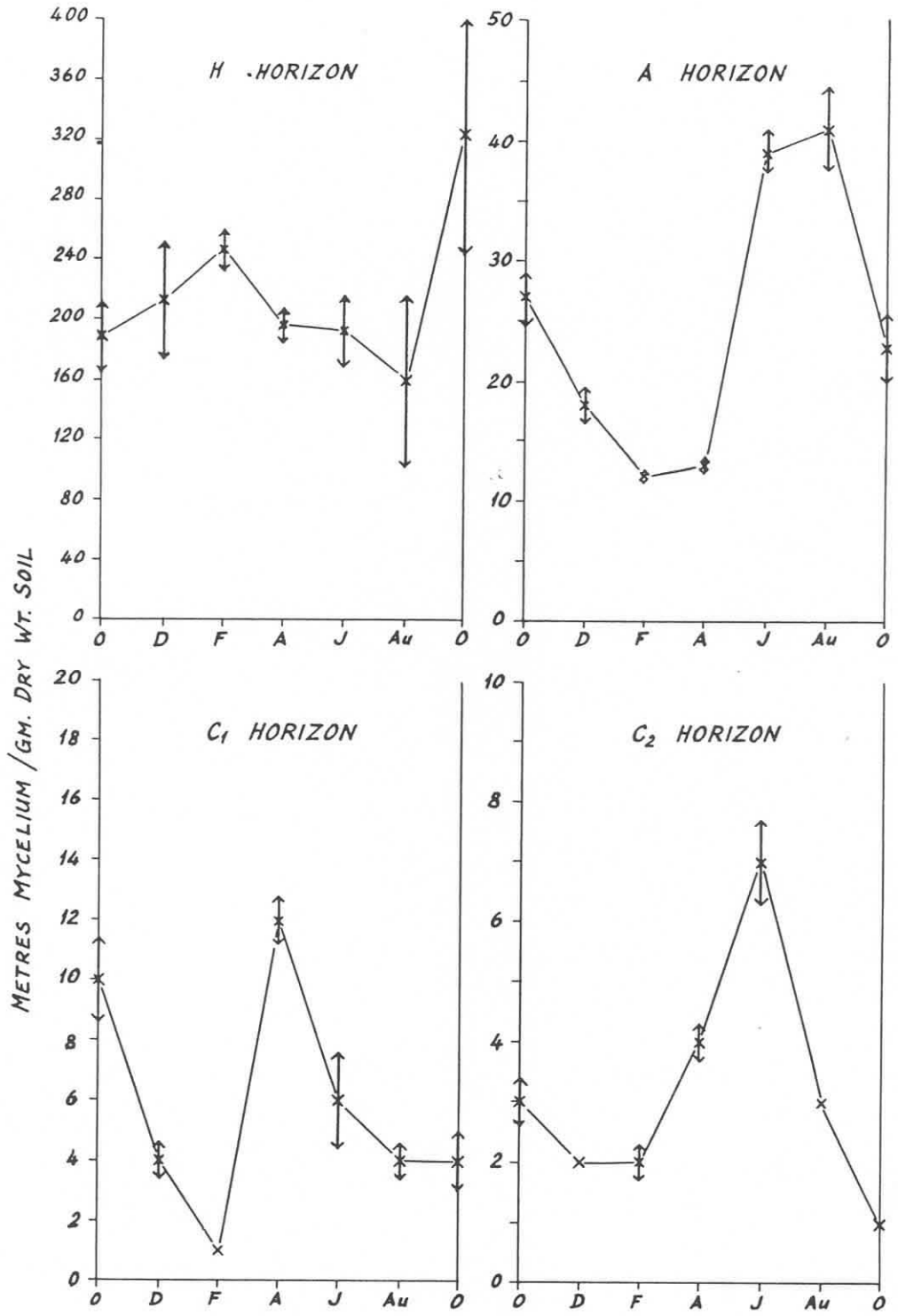

FIG. 1 AND TABLE I

Length of mycelium per gram of oven dry soil in the various soil horizons

Horizon	Length of mycelium (metres)
H	217 ± 67
A_1	25 ± 11
C upper	6 ± 3.7
C lower	3 ± 1.9

The striking difference in mycelial content of the H layer and the joining A_1 horizon is worthy of note, however it should be remembered that the specific gravity of the H horizon is approximately a quarter of that of the mineral horizons. The H layer at Freshfield abounds with dematiaceous hyphal fragments some of which appear empty and dead; since no attempt was made to distinguish between live and dead hyphae the values obtained on lengths of hyphae in the H layer will be magnified (because of the presence of dead hyphal fragments).

TABLE II

Oxygen uptake by samples from different soil horizons based on dry weight and on weight of organic carbon

Horizon	μ 0_2 uptake/gm. dry wt./5 hrs.	% organic carbon	μ 0_2 uptake/gm. org. C_r/5 hrs.
L	471.0	50.0	942.0
F_1	342.5	50.0	685.0
F_2	133.8	46.5	288.0
H	84.2	25.7	327.7
A	6.3	0.63	1000.0
C *upper*	1.9	0.20	950.0
C lower	1.7	0.15	1133.3

Measurement of oxygen uptake.

Rates of oxygen uptake by samples from the various soil horizons are shown in Table 2. These values, based on unit dry weight of soil, represent the means of sets of six determinations carried out at regular intervals throughout one year (thus seasonal variation has been eliminated). These data indicate a progressive decrease in oxygen consumption with increasing depth in the soil (with the litter horizon having much greater « respiratory activity » than the mineral horizons).

It has been suggested (PARKINSON and COUPS, 1963) that respiratory activity of soil samples should be related to the organic carbon content of these soil samples in order to obtain a more accurate idea of the potential activity of the microflora. This relationship is shown, for the Freshfield soil, in Table 2. According to these data, the various horizons can be classed into three groups on the basis of respiratory activity.

1. Group of highest activity (L, A, C_1, C_2) with the C_2 showing the greatest activity per unit weight of carbon.
2. Intermediate group (F_1 layer).
3. Group of lowest activity (F_2 and H layers).

The seasonal variation in respiratory activity together with similar variations in field moisture contents at the times of sampling are shown in Figure 2.

In all horizons, except H layer and lower C (C_2) horizon, there was good correlation between respiratory activity and soil moisture content. In the case of the H layer there was little variation either in respiratory rate or moisture content (perhaps moisture content never became a limiting factor in this layer during the experimental period).

In the litter horizon, the L, F_1 and F_2 layers show maximum respiratory activity in the autumn-winter period (this also being the period of higher moisture content). No noticeable seasonal variation was observed in the other horizons.

DISCUSSION

The inability to distinguish accurately between « active » and « non-active » mycelium is one of the greatest drawbacks to the use of estimates of total mycelial length as an index of fungal activity in soil. The presence of varying proportions of dead mycelium in soil samples will greatly affect results of the measurement of total fungal mycelium as an index of activity and should always be considered during the interpretation of such data.

The progressive decrease in amount of fungal mycelium with increasing depth in the soil has been noted previously, however it should be re-stated that the amount of mycelium in the H layer (this amount being approximately ten times greater than that in the A_1 horizon when calculated on a unit weight basis) is comparatively reduced if calculations are put on a volume basis (when the amount of mycelium in the H layer is only twice that in the A_1 horizon).

Several factors may influence the decrease in total mycelial content down the soil profile. It may be a result of decreased decay rate or increased growth of mycelium in the upper horizons — both the H and A_1 horizons are acidic and this condition may be unfavourable for the activity of mycolytic bacteria, whereas the C horizon is alkaline and the rate of hyphal degradation may be faster. The H horizon contains large numbers of faecal pellets which contain small fragments of mycelia, and, as stated earlier, the high mycelial content of the H horizon may be partially due to the accumulation of dead hyphal fragments.

A greater rate of hyphal growth may be responsible for the higher mycelial content of the upper horizons. Hyphal growth may be governed by a number of factors of which the availability of suitable substrates and suitable moisture content may be two of the most important. In the four horizons studied the H layer was richest in organic matter and also had the highest moisture content. There was a decrease in organic matter content with increasing depth in the soil, but within the C horizon (i.e. comparing the C_1 and C_2) there was little difference in organic matter content.

Soil moisture content varied similarly in the soil profile.

Evidence on seasonal variations in mycelial contents of the horizons is somewhat conflicting. THORNTON (1956) observed maximum mycelial growth in soil during summer, with limited growth during winter. WARCUP (1957) found a great reduction of viable mycelium during the summer, this being

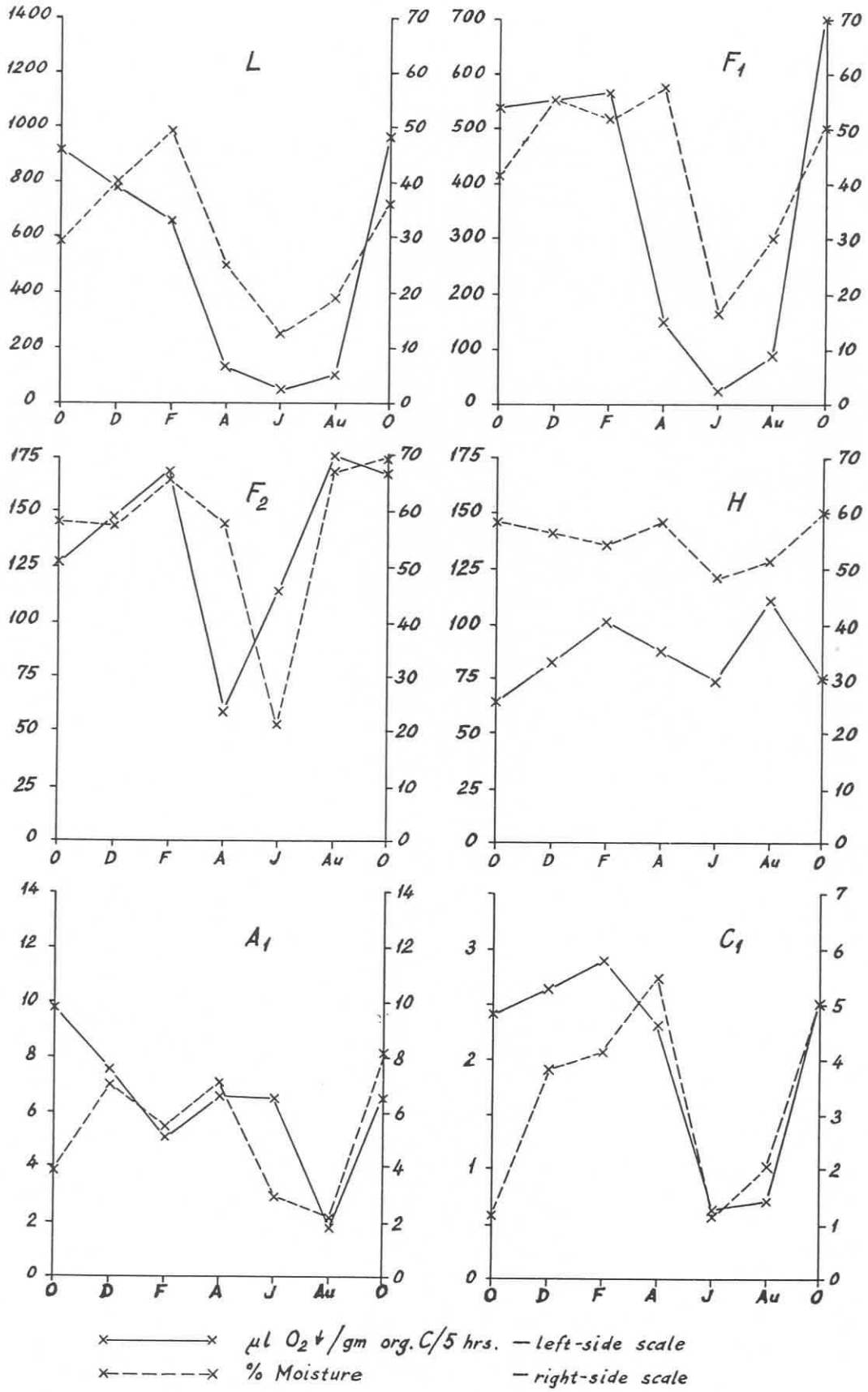
L
F1
F2
H
A1
C1
O D F A J Au O
μl O2↓/gm org. C/5 hrs. — left-side scale
% Moisture — right-side scale

FIG. 2

due mainly to desiccation. WITKAMP (1960) observed a marked increase in the quantity of mycelium during the autumn-winter period. NICHOLAS *et al.* (1965) observed a similar increase in the H layer and A_1 horizon of a podzol under *Pinus sylvestris*, and concluded that the increased litter fall in August might be the main contributory factor for this increase. The results of the present investigation did not show such a marked increase in mycelial development during the autumn-winter period. In contrast with the observations of WITKAMP (1959) and NICHOLAS *et al.* (1965), increases in mycelial lengths were observed during the spring-summer period in all mineral horizons. NICHOLAS *et al.* (1965) did observe a small increase in mycelial activity of all horizons during the summer months.

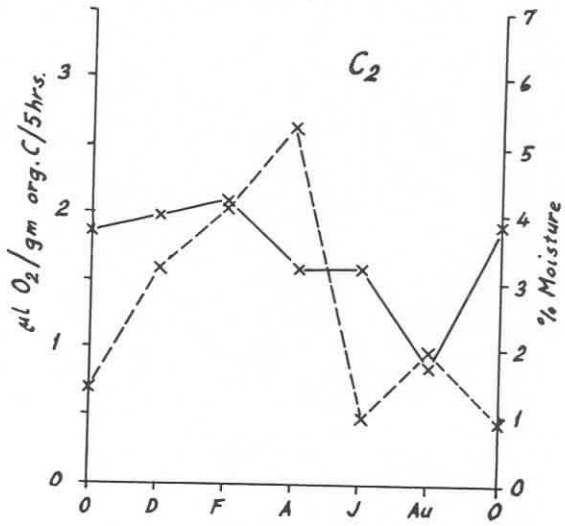

FIG. 2 (CONT.)

The rate of oxygen uptake by unit weights of soil decreased with depth in the soil. However the expression of data in terms of organic carbon provided different data. The sustained increase in respiratory activity in the L, F_1 and F_2 layers during the autumn-winter period may be a result of increased litter fall in the late summer.

One of the most striking observations made during this investigation was the great influence of soil moisture on microbial activity. The data given here show the relation of oxygen uptake of soil samples with soil moisture content. Variations in fungal activity (as indicated by various measurements, i.e. colonization index, spore content, oxygen uptake), both in space and in time, appeared to be governed more by variations in soil moisture content than by any other single factor.

Résumé

Au cours d'une année et à intervalles réguliers, l'étude de la longueur totale du mycelium a été effectué à partir d'échantillons du sol provenant d'une pinède.

L'activité microbienne est ensuite étudiée en mesurant la quantité d'oxygène absorbée par des échantillons de sol.

Les mesures ont été rapportées aux quantités de carbone organique contenues dans les échantillons de sol. Les échantillonnages saisonniers révèlent l'influence de l'humidité du sol sur l'activité microbienne.

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